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The Swedish Patent Office
PCT International Application

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CLAIMS

1. A method for the determination of a tetracycline in a sample characterized in that

- the sample is brought into contact with prokaryotic cells encompassing a DNA vector including a nucleotide sequence encoding a light producing enzyme under transcriptional control of a tetracycline repressor and a tetracycline promoter,
- detecting the luminescence emitted from the intact cells, and
- comparing the emitted luminescence to the luminescence emitted from cells in a control containing no tetracycline
- wherein a detectable luminescence higher than a luminescence of the control indicates the presence of tetracycline in the sample.

2. The method according to claim 1 characterized in that the cells are *Escherichia coli*.

3. The method according to claim 1 or 2 characterized in that the DNA vector is a plasmid containing the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn10*.

4. The method according to claim 3 characterized in that the DNA vector is the plasmid pTetLux1 (SEQ ID NO: 3).

5. The method according to claim 1 or 2 characterized in that

- the DNA vector is a plasmid containing the insect luciferase gene (SEQ ID NO: 1), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn10*, and that

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- D-luciferin is added to the mixture of the sample and the cells in order to initiate the luminescence of the cells.

6. The method according to claim 5 characterized in that the DNA vector is the plasmid pTetLuc1 (SEQ ID NO: 1).

7. The method according to claim 1 ~~any of the claims 1-6~~ characterized in that the sensitivity of the analysis with respect to the tetracycline is controlled by

- increasing or decreasing the concentration of divalent metal ions, e.g. magnesium ions, or
- adjusting the pH, or
- combined adjusting of the divalent metal ion concentration and the pH.

8. The method according to claim 1 ~~any of the claims 1-6~~ characterized in that the sensitivity of the analysis with respect to the tetracycline derivative is increased by the use of cells which are especially antibiotic sensitive mutant strains.

9. The method according to claim 1 ~~any of the claims 1-8~~ characterized in that the luminescence is measured using an X-ray or polaroid film, a CCD-camera, a liquid scintillation counter or a luminometer.

10. The method according to claim 1 ~~any of the claims 1-9~~ characterized in that the sample to be analyzed is milk, fish, meat, infant formula, eggs, honey, vegetables, serum, plasma, whole blood or the like.

11. A recombinant prokaryotic cell characterized in that it encompasses a DNA vector including a nucleotide sequence encoding a light producing enzyme,

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tetracycline repressor and tetracycline promoter, and that the DNA vector is a plasmid containing the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promoter (TetA) (SEQ ID NO: 9) from *Tn10*.

12. The cell according to claim 11 characterized in that it is *Escherichia coli*.

13. The cell according to claim 11 ~~characterized~~ characterized in that it is in dried form, e.g. in lyophilized form.

14. A plasmid characterized in that it comprises the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promoter (TetA) (SEQ ID NO: 9) from *Tn10*.

15. A plasmid according to claim 14 characterized in that it is pTetLux1 (SEQ ID NO: 3).